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SHORT COMMUNICATION

Uncoupling of DNA excision repair and nucleosomal unfolding in poly(ADP-ribose)-depleted mammalian cells

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The repair of DNA damage in eukaryotic cells is closely coupled with local changes of chromatin structure such that newly synthesized repair patches transiently appear in 'free' DNA domains with increased accessibility to enzymatic and chemical probes. We have isolated these domains from mammalian cells repairing bulky DNA adducts. During the first 3 h of repair, excision of adducts occurred exclusively in free DNA and was closely linked with the appearance of newly synthesized repair patches. Following depletion of chromatin-bound poly(ADP-ribose), the repositioning of repair patches into these domains was completely blocked, although overall repair patch synthesis was unaltered. Concomitantly, DNA adducts were no longer excised and tended to accumulate in free DNA domains. Our results suggest a tight coupling of the excision step with the formation of free DNA domains by a mechanism involving poly ADP-ribosylation of chromatin proteins.

In chromatin of mammalian cells, newly synthesized DNA repair patches exhibit a transient micrococcal nuclease hypersensitivity. This hypersensitivity is thought to reflect local disruptions in the tightly packed nucleosomal organization of chromatin, causing exposure of 'free' DNA domains (for review see 1,2). The function of these domains as well as the mechanisms involved in their formation are unknown. We have speculated that the post-translational poly ADP-ribosylation of chromatin proteins might be involved in the formation of free DNA domains in DNA excision repair. Poly ADP-ribosylation is catalyzed by the enzyme poly(ADP-ribose)polymerase (1,3,4; EC 2.4.2.30). Following activation by DNA nicks, this enzyme operates in a strictly processive manner (5). Continuous treatment of living cells with benzamide, a competitive inhibitor of this enzyme (3,4,6), results in the degradation of chromatin-bound ADP-ribose polymers (7) by the enzyme poly(ADP-ribose)glycohydrolase (3,4). Using non-replicating adult rat hepatocytes in primary monolayer culture, we have established conditions for the complete depletion of chromatin-associated poly(ADP-ribose) (7,8). Hepatocytes survive up to 9 days under these conditions and maintain expression of liver-specific functions (8). Thus, poly(ADP-ribose)-depleted hepatocytes represent a convenient model system to study the role of poly ADP-ribosylation in specific steps of DNA repair.

We have previously shown that unfolded free DNA domains can be isolated from the chromatin of intact mammalian cells by taking advantage of their preferential accessibility to 8-methoxypsoralen (9). Upon intercalation into free DNA domains of living cells, 8-methoxypsoralen can be photoactivated to form bifunctional DNA adducts crosslinking the two DNA strands. Crosslinked (free) DNA domains can then be isolated

quantitatively following a denaturation/renaturation treatment and subsequent nuclease S1 digestion of non-crosslinked DNA strands (for details see 9).

Here we have isolated free DNA domains from non-replicating hepatocytes (9), which had been induced to repair bulky DNA adducts by treatment with a low dose of the ultimate carcinogen *N*-acetoxy-2-acetylaminofluorene. Figure 1 shows that newly synthesized repair patches, pulse-labeled for an initial 20 min repair period, gradually accumulate in free DNA domains during subsequent chase periods. This observation is in accordance with previous results based on nuclease probing of chromatin (1,2), or direct isolation of these domains (9).

No repositioning of repair patches into free DNA domains was observed in poly(ADP-ribose)-depleted hepatocytes (7; Figure 1, legend). This depletion did not affect the overall synthesis of repair patches (Figure 1, legend). Thus, the synthesis and repositioning of repair patches in poly(ADP-ribose)-depleted chromatin of hepatocytes were uncoupled.

Figure 2 provides a comparison of excision activity in total chromatin (Figure 2A) and free DNA (Figure 2B) of poly(ADP-ribose)-depleted and undepleted hepatocytes. In the first 3 h of

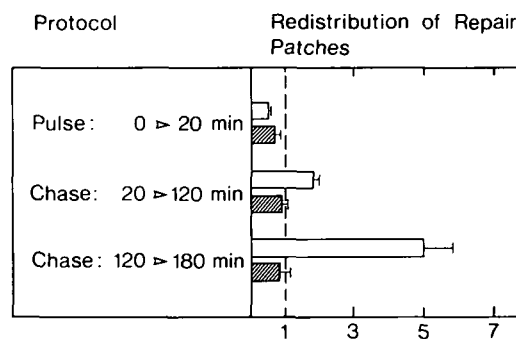


Fig. 1. Redistribution of repair patches relative to free DNA domains in poly(ADP-ribose)-depleted and undepleted hepatocytes. Parenchymal liver cells were isolated from adult rat hepatocytes and cultured for 24 h in the presence or absence of 8 mM benzamide as previously described (9,31). The level of chromatin-bound poly(ADP-ribose) in benzamide-treated cells was < 10 fmol ADP-ribosyl residues/ 10^6 cells, as determined by the method of Jacobson and co-workers (32,33). Hepatocytes were then exposed to $50 \mu\text{M}$ *N*-acetoxy-2-acetylaminofluorene (NCI Chemical Carcinogen Repository, Bethesda, MD, USA) and newly synthesized repair patches were pulse-labeled with [methyl- ^3H]thymidine (49 Ci/mmol, $20 \mu\text{Ci}/3 \text{ ml}$ medium) for 20 min. Incorporation of radioactivity was stopped by two changes of medium containing $1.2 \mu\text{M}$ unlabeled thymidine, followed by a chase in the continuous presence or absence of benzamide. At the end of these incubations, free DNA domains were isolated from hepatocellular chromatin as previously described (9) and analyzed for their content of repair patches (9). The numbers plotted on the abscissa represent the ratios of radioactivity (d.p.m./ μg DNA) incorporated into free DNA to the radioactivity incorporated into bulk DNA. The values reflect the relative accumulation of repair patches in free DNA (9) (a value of 1 indicates no accumulation) and represent the mean \pm SEM of four independent experiments with separate cell preparations. The overall repair incorporation was 511.2 ± 85.4 mmol (controls) and 408.2 ± 94.8 mmol (depleted cells) [methyl- ^3H]thymidine/mol adduct formed (mean \pm SEM, $n = 3$). Black bars: control cells; shaded bars: poly(ADP-ribose)-depleted cells.

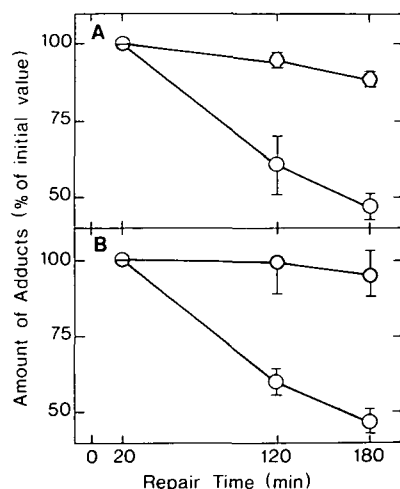


Fig. 2. Excision of deoxyguanosine adducts in total DNA and free DNA domains of poly(ADP-ribose)-depleted and undepleted hepatocytes exposed to *N*-acetoxy-2-acetylaminofluorene (AAAF). Hepatocytes were incubated for 20 min with [G - 3H]AAAF (232 mCi/mmol, total concentration 50 μ M) and the amount of deoxyguanosine adducts was quantified following DNA purification (9). (A) Adduct removal in total DNA; (B) adduct removal in free DNA domains. \circ — \circ , poly(ADP-ribose)-depleted cells; \square — \square , undepleted control cells. The results represent the means \pm SEM of three independent experiments involving separate cell preparations.

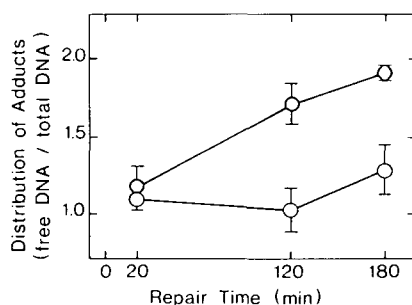


Fig. 3. Relative distribution of the remaining adducts in free DNA domains of poly(ADP-ribose)-depleted and undepleted hepatocytes after various repair intervals. Hepatocytes were incubated with radiolabeled *N*-acetoxy-2-acetylaminofluorene as described in Figure 2 and the deoxyguanosine adduct concentration was quantified in free DNA and expressed relative to their content in total DNA. \circ — \circ , poly(ADP-ribose)-depleted cells; \square — \square , undepleted cells. The values plotted on the ordinate represent the ratios of [G - 3H]AAAF radioactivity in free DNA to the radioactivity contained in bulk DNA. The results represent the means \pm SEM of three independent experiments involving separate cell preparations.

repair, the rate of excision of deoxyguanosine adducts formed during a 20 min incubation of hepatocytes with *N*-acetoxy-2-acetylaminofluorene was almost identical in total chromatin and free DNA. However, no excision of these adducts occurred during the same time period in poly(ADP-ribose)-depleted cells (Figure 2). Hence the repositioning of repair patches (Figure 1) seems to be coupled with adduct excision (Figure 2) by a mechanism involving *de novo* poly ADP-ribosylation of chromatin proteins. This is compatible with the idea that unfolded DNA domains are a preferential site of excision activity (Figure 2).

The results in Figure 3 suggest that some unfolding of chromatin occurs also in poly(ADP-ribose)-depleted cells. A slight accumulation of unexcised DNA adducts in the free DNA fraction was obtained in poly(ADP-ribose)-depleted cells, while no significant accumulation was seen in undepleted control cells. Thus, the unfolded domains formed in poly(ADP-ribose)-depleted

cells are enriched in DNA adducts and largely deficient in repair patches (cf. Figures 1 and 3).

Our results thus reveal a tight functional linkage between structural chromatin rearrangements and the excision step, and dissect the process of repair patch synthesis from the patch repositioning relative to nucleosomally organized chromatin regions. Moreover, our results tentatively identify a requirement for poly ADP-ribosylation in co-ordinating excision repair with structural rearrangements of chromatin. The role of this post-translational protein modification is poorly understood, but all protein acceptors of poly(ADP-ribose) hitherto identified (for review see 4) share the capacity to bind to nucleic acids. Poly ADP-ribosylation of these proteins *in vitro* reversibly alters their DNA binding affinity (10–15). Enzymatic addition and removal of ADP-ribose polymers on chromatin preparations *in vitro* induces reversible relaxation of polynucleosomes (16), the relaxation state being directly related to the size of histone-bound ADP-ribosyl polymers (17–19). Histones are also a physiological target of ADP-ribose modifications in carcinogen-treated mammalian cells *in vivo* (20,21). Thus, the reversible modification of DNA–protein interactions (14,15,22,23) by protein ADP-ribosylation may be the underlying molecular mechanism for the transient formation of free DNA domains in eukaryotic excision repair.

As far as our findings ascribe poly ADP-ribosylation a role in DNA excision repair, they contrast the hypothesis of Shall and associates who envisioned poly ADP-ribosylation as a direct regulatory mechanism of ligation activity in the repair of alkylation damage in DNA (24,25). However, other reports could not confirm such a role for poly(ADP-ribose) in DNA excision repair (26–30). This contradictory phenomenology may be reconciled on the premise that poly ADP-ribosylation affects local disruptions of chromatin structure and this secondarily affects DNA repair reactions (4,26–30).

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